(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 July 2002 (25.07.2002)

PCT

(10) International Publication Number
WO 02/056823 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/IL02/00051

(22) International Filing Date: 18 January 2002 (18.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
140970 18 January 2001 (18.01.2001) IL

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(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), DE (utility model), DK (utility model), DM, DZ, EC, EE (utility model), ES, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/056823 A2

(54) Title: REDOX THERAPY FOR TUMORS

(57) Abstract: A method for treating malignancies and/or otherwise controlling the growth and/or proliferative behavior and/or other biological functions of a cell displaying malignant properties, through the control of the redox state or environment of the cell, preferably through the administration of a GSH-decreasing agent.

REDOX THERAPY FOR TUMORS

FIELD OF THE INVENTION

The present invention is of a treatment for defective cells, such as tumor or malignant cells, by altering the redox state or environment of the cell, and in particular, of such a treatment in which the balance of GSH (glutathione) to GSSG (glutathione disulfide) is altered.

BACKGROUND OF THE INVENTION

The redox state or environment of a cell refers to the balance between oxidative processes and reducing processes. The energy released by oxidative processes is used by the cell to build cellular and tissue structures, and to operate and maintain such structures. The term redox state has typically been used to refer to two molecules between which electrons may be traded, and which are referred to as a "redox couple". One example of such a couple is the two molecules GSH and GSSG, which help to determine the balance between oxidative and reducing processes, and hence the redox state or environment of the cell. This couple is actually GSSG / 2GSH. The presence of the balance between these couples may have many important biological effects, particularly with regard to the growth and general proliferative behavior of the cell.

Without wishing to be limited to a single mechanism, the induction of the cessation of cell proliferation, and also control over proliferative cell behaviors, appears to be related to the redox state of the cell, as explained in greater detail below.

One way to describe the redox state or environment of the cell is through the Nernst Equation. The intracellular redox state of the cell, E, is, according to the Nernst equation, a function of log $\{[GSSG]/[GSH]^2\}$, where [GSH] and [GSSG] are the concentrations of glutathione and its oxidized form, respectively. As GSH decreases, E increases (Hutter et al., 1997).

Decreasing the level of GSH increases the redox state of the cell, and results in a reduction of cell proliferation. Normal actively proliferating (foreskin) fibroblasts have an E of, –222mV, which is about 10 mV lower than that of neoplastic fibrosarcoma cells, where E is about –211 mV. (Hutter et al. 1997). The display of

proliferative behavior appears to be associated with the redox state of the cell. It is shown that decreasing the level of GSH increases the redox state of a cell, and results in a decrease or cessation of cell proliferation. Again, without wishing to be limited to a single mechanism such behavior may be at least partially induced through effects on the retinoblastoma (RB) protein.

When the GSH in NK3.3 cells is decreased, E is increased, the retinoblastoma (RB) protein in NK3.3 cells cannot be phosphorylated and these cells cease to proliferate. Dephosphorylated RB traps the transcription factors that generate the cyclins required for cell proliferation, resulting in a cyclin-poor cell. When GSH is restored, E is decreased, RB can be phosphorylated and these cells proliferate (Yamauchi et al., 1997). This critical value of E which induces cessation of cell proliferation (CCP), is designated E_{ccp}. If this arrest in the cell cycle prevents the cell from entering into phase S or subsequent phases of the cell cycle, apoptosis is induced. Thus, CCP is manifest as either cell cycle arrest or apoptosis or cytotoxicity

It has been shown in the publication of Hutter et al (1997) that adjusting the values of [GSH] also changes E. It can be calculated from Table 1 in their publication that the value of E that corresponds to cessation of cell proliferation (E_{ccp}) of fibroblasts is –205 mV. Below E_{ccp}, the cells proliferate; above E_{ccp}, cells do not proliferate and the cell density decreases up to more than 50%.

Lee et al. (1998) showed that glucose deprivation of human breast carcinoma cells induced CCP, which can lead to apoptosis. They also measured the changes in [GSH] and [GSSG]. They reported that, although there was a large spread in the data, there was a definite difference between the test and control. The changes in the mean values of [GSH] and [GSSG] in these cancer cells produced by glucose deprivation, correspond to 3mV. As E of cancer cells is about –211 mV, then, if the mean value of the change in E is applied, E_{ccp} = +3 –211 or about –208 mV. Thus, similar to the analysis of the results of Hutter et al. (1997), here also, artificially increasing E to E_{ccp}, induces CCP.

Rossi et al. (1986) decreased the [GSH] in normal hepatocyte cells. The data in their publication showed (Table VI-last three entries) that some degree of CCP (cytotoxicity) was induced after 3 hours, when the decrease in [GSH] reached 58% of its original value. According to the Nernst equation, this decrease in GSH

concentration, due to formation of a Michael Adduct (Cornwell et al.,1998 and Thornton et al., 1998), corresponds to an increase of about 15 mV. If the E of normal actively proliferating cells is –222mV (Hutter et al., 1997), E_{ccp} is about –207 mV. However, significant cytotoxicity was attained only when the decrease in GSH was 90 % or more of its original value. Here again, artificially increasing E to E_{ccp} results in CCP.

Tumor Treatment

There have been many approaches to treating tumors. Some of these approaches are relatively selective, such as the surgical removal of the tumor. In general, surgery is effective if the tumor has not spread and all the malignant cells have been removed. Other approaches are less selective and include radiation and chemotherapy, which frequently effect normal cells. An agent is considered to provide a selective result if it mostly affects the cancer cells of the tumor, but does little, if any, harm to the adjacent normal cells of the tissue.

Many of the classical chemotherapeutic agents are usually more effective when the cancer cells in the tumor are rapidly proliferating. These cytotoxic agents, such as those listed in Table 1a, generally affect DNA during cell proliferation, primarily killing cancer cells rather than the relatively slowly proliferating normal cells (Smaaland et al., 1991). But this selectivity factor is not operative when treating slowly proliferating cancer cells. Newer anti-cancer agents have been developed such as those listed in Table 1b. Various mechanisms have been suggested for these different agents listed in Tables 1a and 1b, hereby designated as standard chemotherapeutic agents. However, there still exists uncertainty about the precise mechanisms involved. The concentrations of these cytotoxic drugs are usually limited to less than 5 μM (Ramachandran et al., 1999) in order to minimize injury to normal cells.

Reactive oxygen species (ROS), as generated by radiation, for example, are believed to cause mutations that produce cancer. There appears to be a consensus that anti-oxidants (e.g. GSH) which can scavenge / neutralize the ROS, are required to prevent and cure cancer (Dai et al., 1999, Sen et al., 1999).

The background art thus clearly teaches that anti-oxidants are required to treat tumors. The background art also teaches that the concentration of such agents should be below 5 micromolar (Ramachadran et al., 1999). A number of compounds have been shown to be successful for treating tumor cell lines (see examples below and Table 2), yet the basic mechanism of how these various compounds work remains unclear, as can be seen from the results presented below. Clearly, some of these agents are not antioxidants.

The examples below are illustrative of the many recently published reports in the art. Most, if not all, involve experiments with cell lines, which intrinsically involve relatively rapid cell proliferation. Hence the results with these various agents do not demonstrate their selectivity and /or effectiveness in slowly growing tumors.

Dai et al. (1999) introduced As_2O_3 into various cell lines. The intracellular glutathione GSH content had a decisive effect on the induction of apoptosis, the sensitivity to apoptosis increasing as the GSH content of the cell decreased. GSH forms an adduct with As, viz., $As(GS)_3$. These researchers experimentally varied the GSH content of the various cells with BSO (buthionine sulfoximine), which inhibits a key enzyme in GSH synthesis (gamma-glutamylcysteine). The sensitivity to apoptosis increased with decreasing GSH content. By itself, BSO, which caused a decrease in GSH content of about 70% in the cell, did not induce significant apoptosis. Normal cells were the least sensitive to apoptosis.

Nicole et al. (1998) showed that the introduction of BSO to neuroblastoma cells, decreasing their GSH content by 98%, induced apoptosis. They concluded that with these cells, there was a cause/effect relationship between decreasing GSH and apoptosis induction.

Sen et al. (1999) introduced alpha-lipoic acid into both Jurkat T-cell leukemia cells and normal lymphocytes. The former underwent apoptosis, whereas the latter did not. They suggested that the induction of apoptosis by alpha-lipoic acid was because this acid is a sulfur-containing antioxidant that provides strong reducing power and leads to the reduction of protein thiols.

Lizard et al. (1998) reported that the introduction of 7-ketochlosterol to U937 cancer cells induced apoptosis. They found that apoptosis was enhanced by the addition of BSO and inhibited by the addition of NAC (N-acetyl-L-cysteine). (NAC is a cysteine precursor which penetrates the cell and is converted to cysteine by

deacetylation, and is a GSH precursor.) These researchers suggested that oxidative processes are involved in 7-ketochloresterol -induced cell death.

Rudra et al. (1999) reported that the introduction of acrolein induced cytotoxicity in various cancer cell lines, such as A-427 and A-172. They demonstrated that the sensitivity to growth inhibition increases as GSH decreases. They also reported that A-427 is highly sensitive to docosahexaenoic acid, and that acrolein potentiates the cytotoxic effect of this acid. These researchers reported that acrolein depletes thiols and is highly toxic to both normal human bronchial fibroblasts and human bronchial epithelial cells in the respiratory system.

Rossi et al, (1986), Thornton et al. (1995) and Cornwell et al (1998), introduced various quinones or quinone precursors to both normal cells, such as smooth muscle and hepatocytes, and to leukemic cells. Rossi et al. (1986) concluded that when GSH decreased by 90-95% of the original amount in the hepatocytes, significant cytotoxicity was induced. They all conclude that the quinones formed a Michael Adduct with the GSH.

Ramachandran et al. (1999) introduced curcumin to both human mammary epithelial cells (MCF-10A) and breast carcinoma (MCF-7/TH) cell lines. They concluded that the induction of apoptosis is due to the effect of the curcumin on some of the genes associated with cell proliferation.

Zhou et al. (1998) introduced soy isoflavones to human prostate carcinoma cells and normal vascular endothelial cells. They suggested that these soy products inhibit experimental prostate tumor growth through a combination of direct effects on tumor cells and indirect effects on tumor neovasculature.

Paschka et al. (1998) induced apoptosis of prostate cancer cell lines by introducing green tea phenols including (-)-epigallocatechin-3-gallate.

The concentration of the compounds required to induce cytotoxicty /apoptosis in the above nine cases were on the order of a few micromolar to tens of micromolar. This is considered too high, as the concentration of the standard chemotherapeutic agent should be less than 5 micromolar (Ramachadran et al., 1999). Furthermore, as stated above, there is no evidence that any of these agents are effective and/or selective on slowly growing tumors.

With respect to tumors in general, especially slowly growing tumors, there is a dire need for agents that can cause the selective cessation of cell proliferation (CCP),

either as a result of cell arrest or apoptosis, similar to the effect of radiation on cells. Radiation induces p53, which, in turn, induces p21, which combines with/inactivates the cyclins required for cell proliferation. The result of the cell becoming effectively cyclin-poor is cell cycle arrest or apoptosis (Gottleib & Orens, 1996); i.e. CCP. This mechanism is independent of the rate of cell cycle proliferation. However, in many cases, radiation is not adequately selective, and in addition it causes problematic side effects. Thus, more selective and effective treatments are clearly required.

BRIEF DESCRIPTION OF THE TABLES

The foregoing and other objects, aspects and advantages will be better understood from the following detailed description of a preferred embodiment of the invention with reference to the tables, wherein:

Table 1a tabulates classical chemotherapeutic agents for use with the present invention;

Table 1b tabulates new chemotherapeutic agents for use with the present invention; and

Table 2 tabulates other GSH depletion agents for use with the present invention.

SUMMARY OF THE INVENTION

The background art does not teach or suggest agents, or their effective concentrations thereof, that can cause the selective cessation of cell proliferation. The background art also does not teach or suggest a method for treating malignancies on the basis of altering redox potential in the cell.

The present invention overcomes these deficiencies of the background art by providing a method for treating malignancies and/or otherwise controlling the growth and/or proliferative behavior and/or other biological functions of a cell displaying malignant properties, through the control of the redox state or environment of the cell.

The present invention specifically teaches away from the background art, as the present invention teaches that oxidants should be used for tumor treatment, so that the damage to the DNA is not the cause of cell death, but rather cell death is the result of the cell undergoing apoptosis, which ultimately damages the DNA. The present invention also teaches the use of a high concentration of anti-tumor agents, which

again teaches away from the background art. The present invention also teaches that certain of the effects of radiation on cells, resulting in CCP, which may lead to apoptosis, can be obtained effectively and selectively on human cancer cells, ideally, by introducing and maintaining a well poised redox buffer, set a few mV above E_{ccp} (e.g. at about -200mV) to a cancer tissue; optionally and more preferably, by administering GSH-depleting agents in doses of up to several grams per day.

The description below is of redox tumor treatment to induce CCP of slowly growing tumor cells selectively. Although slowly growing, these tumor cells are growing too rapidly, relative to adjacent normal cells.

The present invention also encompasses the treatment of diseases in which cells are dying too rapidly such as Alzheimers disease and Parkinsons disease. For such diseases, artificially decreasing E below E_{ccp}, preferably by increasing [GSH], may optionally be used to retard apoptosis and/or enhance proliferation.

Throughout this specification, various scientific publications are referenced. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosure of all these publications in their entireties are hereby incorporated by reference into this specification in order to more fully describe the state of the art to which this invention pertains.

The term "tumor" as used herein encompasses all types of tumors including solid and non-solid tumors and including, *inter alia*, melanoma, carcinoma, lymphoma, leukemia, blastoma. The term "tumor" encompasses primary tumors, secondary tumors, and metastases thereof in the same organ or in another organ. It is envisaged that this invention will work in tumor cells in which the RB protein is operative.

The terms "treatment of a tumor" and "anti-tumor" as used herein refer to a treatment or a composition which retards the proliferation of a tumor and /or causes regression of a tumor. A pharmaceutically effective amount of a GSH-decreasing agent is an amount of a GSH-decreasing agent which retards the proliferation of a tumor and /or causes regression of a tumor. The combined amount of GSH-decreasing agents which are pharmaceutically effective is an amount of two or more GSH-decreasing agents which when combined retard the proliferation of a tumor and /or cause regression of a tumor.

DETAILED DESCRIPTION OF THE INVENTION

The present invention involves preferably, the use of a combination of GSH depleting agents , or their precursors, which, in the recommended dosages, are not cytotoxic in the sense that these agents do not directly damage the DNA of the cells, but rather, these agents induce cessation of cell proliferation, CCP. Examples of precursors that metabolize in the body to form GSH-depleting agents are tocopherols that form quinones (Rossi et al., 1986, Thornton et al., 1995, and Cornwell et al., 1998) and polyunsaturated fatty acids (PUFA), which form alpha- and beta-unsaturated aldehydes (Esterbauer et al., 1991). As noted above, these GSH-depleting agents generate an increase in the intracellular redox state, E, which inhibits phosphorylation of the RB protein, thereby providing a cyclin-poor cell. The dephosphorylated RB protein continues to trap the transcription factors required by the cell to form the cyclins, which are required to progress through the cell cycle. The cell becomes, effectively, cyclin poor, inducing CCP. If the cell cannot enter S or a subsequent phase of the cell cycle, apoptosis is induced, and this will generate DNA damage.

The approach of the present invention has a built-in selectivity. As normal proliferating cells generally have a lower E value, that is, more GSH than cancer cells (e.g. Hutter et al.), adding a limited amount of GSH-decreasing agents to a tissue containing a tumor can increase the E of the cancer cells to E_{ccp} or beyond, whereas the E value of normal proliferating cells in the tissue can still remain lower than E_{ccp}, as described previously.

Until the present invention, it has not been recognized that there is an underlying mechanism common to most of the above experiments that resulted in CCP; i.e, there exists a critical intracellular redox state or redox potential, E_{ccp}, and that agents that decrease GSH concentration will increase E above E_{ccp}, inhibiting RB phosphorylation, inducing CCP, which can lead to apoptosis.

Thus, the teaching of this invention is contrary to the background art. The background art teaches that antioxidants should be used to both prevent and to treat tumors (e.g. Sen et al., 1999). This invention teaches that agents that are effectively pro-oxidants are required to treat tumors, where a pro-oxidant is defined as an agent that increases the intracellular redox state or redox potential to a more oxidizing value,

i.e., a higher value of E. This includes classical oxidizing agents, such as oxygen or hydrogen peroxide, or Michael Acceptors such as quinones or alpha- and beta-unsaturated aldehydes or their precursors.

EXAMPLE 1

CLASSIFICATION AND USE OF GSH DEPLETING VEHICLES

GSH depleting vehicles (which are agents or procedures that increase the redox potential of the cell, E, by decreasing [GSH] or concentration of GSH in the cell) are therefore effectively pro-oxidants. These agents can be classified as follows:

1) Oxidation of GSH to GSSG by oxidizing agents, or their precursors

Non-limiting, illustrative examples of suitable agents include:

1. Alpha-lipoic acid (Sen et al., 1999)
2. Hydrogen peroxide (Rimpler et al., 1999)
3. Ascorbic acid (auto-oxidized) precursor of (2) (see Dai et al., 1999)
4. Dopamine {auto-oxidized} (Kang et al., 1998)
5. Oxidized low density lipoproteins (LDLs) (Kinscherf et al. 1997)
6. Quinones, especially fully substituted, such as duraquinone (Rossi et al., 1986)

2) Adduct Formation : combination of GSH with a Michael Acceptor agent comprising a C=O conjugated to a C=C, or their precursors, to form a Michael Adduct; also combination via Nucleophilic addition to GSH.

Michael adducts are formed from a combination of a Michael Acceptor, which is an electrophile, and GSH, which is a nucleophile.

Non-limiting, illustrative examples of suitable agents include:

1. Incompletely substituted quinones (Rossi et al., 1986)
2. Alpha- and beta- unsaturated aldehydes (Rudra et al.,1999)
3. Precursor of example (1); e.g. tocopherols (Thornton et al.,1995)

4. Precursor of example (2); e.g. polyunsaturated fatty acids(Esterbauer et al., 1991)

3) Other Types of Adduct Formation

Non-limiting, illustrative examples of suitable agents for forming other types of adducts include: Inorganic Salts; e.g. arsenic trioxide to form $As(GS)_3$ (Dai et al., 1999).

4)Inhibitor of GSH or General Protein Synthesis

These agents act to inhibit GSH synthesis specifically, or protein synthesis generally. Non-limiting, illustrative examples of suitable agents include:

1. Buthionine Sulfoximine {BSO} (Nicole et al., 1998)
2. Glucose deprivation (Lee et al., 1998)
3. Cycloheximide (Zetterberg et al. 1985,1995)
4. Hypoxia (Araye et al, 1998)

In addition to, or as a replacement for, the administration of the above-referenced agents, various procedures may optionally be performed that generate reactive oxygen species (ROS) that may oxidize GSH to GSSG. Non-limiting, illustrative examples of suitable procedures include:

1. Radiation(Gottlieb and Oren, 1996)
2. Hypothermia (Lord-Fontaine and Averill, 1999)

Note that since tumors comprise cancer cells that have mutations, adding any of the above agents might also enhance the synthesis of GSH in these defective cancer cells, as well as oxidize or form an adduct with the GSH, so that the net result might be an net increase in the steady-state concentration of GSH. Without wishing to be limited to a single hypothesis, the present invention optionally and preferably includes the control of the steady-state concentration of GSH, rather than some instantaneous (and possibly temporary) change in GSH level as a consequence of adding an agent or performing a procedure. Only when the steady-state concentration of GSH decreases, can E increase to beyond E_{ccp}.

Table 2 lists non-limiting, illustrative examples of various agents /compounds, which, as they are, or as one of their metabolic products, can form an adduct with GSH.

EXAMPLE 2

General Approaches for implementing the subject invention

8. Combination of GSH-depleting agents and / or procedures from the above list.

Each of the previously described agents, methods or categories has unique kinetics; for a given concentration, both the effective time to decrease GSH, and the resultant steady-state value of GSH, are different in each case. Their combination is synergistic, providing the ability to attain the optimum rate of decrease and optimum value of the steady-state concentration of GSH.

II. **Combination of GSH-depleting agents that are water soluble with lipid soluble agents.** By lipid soluble agent, it is meant substances containing an aliphatic side chain or otherwise being lipid soluble. Preferably, the method of the present invention comprises the administration of water-soluble GSH-depleting agents with lipid soluble GSH-depleting agents from the above categories. This enhances the chances that some GSH-depleting agents gain access to different parts of the body characterized by different "solubility barriers". Thus, no matter where a particular GSH-depleting agent is located or formed, for example, the stomach, or elsewhere in the body, there is a good chance the GSH-depleting agents will reach to the tumor. Of course, this solubility problem can also optionally be overcome if the GSH-depleting agents are introduced directly into the tumor tissue, for example, by injection into the prostate sac.

III. **Combination of standard chemotherapeutic agents with I and/or II.**

A standard chemotherapeutic agent such as melphalan attacks the DNA via alkylation or intercalation and hence kills proliferating cells. GSH detoxifies standard chemotherapeutic agents, such as melphalan (Dai et al., 1999). Hence, GSH-depleting agents are effectively, "sensitizing agents" which will enhance this standard

chemotherapeutic agent attack on the DNA of the cell, restoring cytotoxicty of drug-resistant cells (e.g. to melphelan). The addition of GSH-depleting agents, in effect, weakens the tumor cells selectively, so that a smaller concentration of a standard chemotherapeutic agent will be rendered more effective against tumor cells.

Regardless of the particular agent or procedure, or combination thereof, according to a preferred embodiment of the present invention, the intracellular redox state of the cell, E, is preferably increased beyond a critical value, E_{ccp}, to induce apoptosis. E can be increased passively by decreasing the free GSH content of the cell; for example by inhibiting GSH synthesis. GSH can be decreased actively in various ways. For example, by introducing classical oxidizing agents (pro-oxidants), such as H_2O_2, oxygen, fully substituted quinones, alpha-lipoic acid, and others, which can oxidize GSH to GSSG. Alternatively, agents that combine with GSH to form an adduct also increase E of the cell actively, and hence are, effectively, oxidizing agents. These adduct forming agents produce the same general effect as the introduction of an oxidizing agent; i.e. they decrease GSH and increase E.

However, antioxidants are reducing agents which increase GSH and decrease E; i.e. antioxidants allow the RB protein to remain/become phosphorylated, allowing cell proliferation. Thus, whereas antioxidants might prevent cancer (e.g. by scavenging / neutralizing reactive oxygen species - Dai et al., 1999), antioxidants will enhance proliferation of cancer (and normal) cells.

The novel approach of the present invention, which, without wishing to be limited to a single hypothesis, applies the anti-proliferative effect of the RB protein to halt the progress of the cell through its cycle by increasing E, should be useful for any cancer having an operational RB protein.

Table 2 lists various GSH-depleting agents for optional use with the present invention.

EXAMPLE 3

PREFERRED EMBODIMENTS OF THE PRESENT INVENTION

One embodiment of the invention is to use a single GSH-decreasing agent. Depending on the solubility barrier associated with the tumor, *lipid or aqueous (see above),* a GSH-decreasing agent with an aliphatic side chain (e.g. gamma- and delta-

tocopherol quinones (Thornton et al.1995, Cornwell et al., 1998), or without (e.g. quinone - see Rossi et al., 1986) respectively, should be chosen. Compounds with aliphatic side chain groups are lipid soluble. If the tumor is in the brain, the GSH-depleting agent should preferably be a relatively small molecule to optimize its passage through the blood-brain barrier, e.g. hydrogen peroxide or its precursor (Dai et al. 1999) One preferred feature of the present invention is that the single GSH-depleting agent is "matched" to the location of the specific non-metastasizing tumor. Another preferred feature of the present invention is the higher dosage of the agent or agents which are used.

Another preferred embodiment of the invention, especially if the tumor has spread, is to use more than one GSH-decreasing agent, perhaps including one with and one without an aliphatic side chain, to optimize access to different parts of the body.

Yet another preferred embodiment of the invention is to use more than two GSH-decreasing agents, again with and without an aliphatic side chain, from the adduct and oxidation classes of GSH-decreasing agents. As adduct formation of GSH requires *de novo* synthesis of more GSH, to restore what has been removed, adduct formation is (presumably) longer lasting. Furthermore, the kinetics of oxidation are not the same as adduct formation; oxidation generates GSSG, which, according to the Nernst equation, amplifies the increase in E (amplifies the increase in the ratio of $[GSSG]/[GSH]^2$ so that not only does the denominator decrease, but the numerator also increases). Thus, a combination of an agent that oxidizes GSH, together with an agent that forms an adduct with GSH, provides a synergistic effect. This combination produces both a more rapid attainment of E_{ccp}, and maintains it over a longer period of time, than either single agent separately.

Still another preferred embodiment of the invention is to use two different pairs of agents, one pair from the oxidation class, another from the adduct class of GSH-decreasing agents, where one of each pair has an aliphatic side chain, and the other member of the same pair lacks an aliphatic side chain.

Another preferred embodiment is to combine a cytotoxic substance at a concentration below its toxic level with any of the above embodiments. Examples are inhibitors of GSH synthesis such as BSO , cycloheximide, or standard chemotherapeutic agents (see Table 1), such as melphelan.

Still another preferred embodiment is to combine glucose deprivation with any

of the above (A) to (E). This is a very safe method of decreasing protein and other synthesis. This invention teaches that this approach, viz., effective starvation to some degree of cancer cells via an inhibitor of GSH synthesis, renders these cancer cells more vulnerable to damage by standard chemotherapeutic agents. In effect, very low dosages (less than 5μM) of standard chemotherapeutic agents will selectively kill only the cancer cells, if combined with an inhibitor of GSH synthesis.

In general, if the cancer (tumor) cells are insensitive to the standard chemotherapeutic agent(s) being used, any of the above may be expected to restore the sensitivity of the cancer cells to cell death.

EXAMPLE 3

FORMULATIONS AND METHODS OF USE

This Example refers to different formulations for administering an agent or agents for treating a tumor for performing the method of the present invention and/or for forming a composition for use according to the present invention. The terms "treatment of a tumor" and "anti-tumor" as used herein refer to a treatment or a composition which retards the proliferation of a tumor and /or causes regression of a tumor.

The composition preferably includes a pharmaceutically effective amount of a GSH-decreasing agent. A pharmaceutically effective amount of a GSH-decreasing agent is an amount of a GSH-decreasing agent which retards the proliferation of a tumor and /or causes regression of a tumor. The combined amount of GSH-decreasing agents which are pharmaceutically effective is an amount of two or more GSH-decreasing agents which when combined retard the proliferation of a tumor and /or cause regression of a tumor.

A formulation for performing the method according to the present invention and/or for forming a composition for use according to the present invention may optionally and preferably include a carrier with the agent or agents. As used herein, the term "carrier" encompasses any of the standard pharmaceutical carriers. Such carriers are well known in the art and may include, but are in no way and are not intended to be limited to, any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, emulsions such as oil/water emulsion, suspensions, and various types of wetting agents. Typically, such carriers contain

excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives, preservatives and the like, or other ingredients.

Compositions (medicaments) comprising such carriers are optionally formulated by well-known conventional methods. The compositions of this invention may include sterile solutions, tablets, coated tablets, capsules, pills, ointments, creams, lotions, gels, suppositories, drops, liquids, sprays and powders or any other means known in the art.

A drug delivery system embodying the present invention optionally and preferably comprises a pharmaceutical package having at least one, and preferably two or three or four or more separate dosage units of different GSH-decreasing agents.

As regards dosage, the GSH-decreasing agent is optionally and more preferably administered in an amount of from about 0.1 to about 50 mg/Kg body weight per day, preferably from about 10 to about 45 mg/Kg body weight per day, most preferably from about 20 to about 40 mg/Kg body weight per day. Thus an adult dosage of GSH-decreasing agent(s) may optionally be as much as about 2 grams per day or more.

The administration of the compositions of this invention may be effected by any of the well-known (and/or other suitable) methods of administration, including, but not limited to, intravenous, intramuscular, intravesical, intraperitoneal, topical, subcutaneous, rectal, vaginal, ophthalmical, pulmonary (inhalation), nasal, oral and buccal administration, by inhalation or insufflation (via the nose or mouth) or by administration as a coating to a medical device (e.g. a stent).

When a second GSH-decreasing agent is administered in conjunction with a first GSH-decreasing agent this means that the second GSH-decreasing agent is administered prior to, at the same time as, or subsequent to, administration of the first GSH-decreasing agent, preferably as prescribed by a treatment schedule. Similarly, when a third GSH-decreasing agent is administered in conjunction with first and second GSH-decreasing agents this means that the third GSH-decreasing agent is administered prior to, at the same time as, or subsequent to ,administration of the first and second GSH-decreasing agents. Similarly, when a fourth GSH-decreasing agent is administered in conjunction with first, second and third GSH-decreasing agents this

means that the fourth GSH-decreasing agent is administered prior to, at the same time as, or subsequent to, administration of the first, second and third GSH-decreasing agents. It is also envisaged that 5 or more GSH-decreasing agents may optionally be used in an embodiment of this invention.

One preferred embodiment of this invention is a method of treating a subject suffering from a tumor which preferably comprises administering a pharmaceutically effective amount of a GSH-decreasing agent to the subject. In different embodiments, the GSH-decreasing agent contains or does not contain an aliphatic side chain .

In a preferred embodiment, the GSH-decreasing agent containing the aliphatic side chain is a first GSH-decreasing agent and a second GSH-decreasing agent is administered in conjunction with the first GSH-decreasing agent, and the combined amount of GSH-decreasing agents is a pharmaceutically effective amount. In another preferred embodiment the first GSH-decreasing agent harbors a aliphatic side chain and the second GSH-decreasing agent does not harbor a aliphatic side chain.

In another preferred embodiment of the method, the first GSH-decreasing agent harboring the aliphatic side chain is selected from the group consisting of alpha -tocopherol quinone, gamma- tocopherol quinone, delta- tocopherol quinone and coenzyme Q (ubiquinone) and the second GSH-decreasing agent which does not harbor a aliphatic side chain is selected from the group consisting of those chemicals which do not harbor a aliphatic side chain which are listed in Table 2 and alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

In another preferred embodiment of the method, the first and second GSH-decreasing agents are from the adduct formation class of GSH-decreasing agents, and in a particularly preferred embodiment the first and second GSH-decreasing agents from the adduct formation class of GSH-decreasing agents are selected from the group consisting of the chemicals listed in Table 2.

In another preferred embodiment of the method, a third GSH-decreasing agent is administered in conjunction with the first and second GSH-decreasing agents, and this third GSH-decreasing agent is preferably from the oxidation class of GSH-decreasing agents, more preferably selected from the group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

In another preferred embodiment of the method a fourth GSH-decreasing agent is administered in conjunction with the first, second and third GSH-decreasing agents, and preferably the third and fourth GSH-decreasing agents are from the oxidation class of GSH decreasing agents, most preferably selected from the group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine {auto-oxidized}, oxidized low density lipoproteins (LDLs), quinones and duraquinone.

In another preferred embodiment of the method, at least one of the GSH-decreasing agents is selected from the group consisting of foods, spices and vitamins, preferably selected from the group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone. In other preferred embodiments of the method, at least two, at least three and at least four of the GSH-decreasing agents are selected from the group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

In another preferred embodiment of the method one of the GSH-decreasing agents is buthionine sulfoximine or cycloheximide. In another preferred embodiment of the method buthionine sulfoximine or cycloheximide is administered in conjunction with the GSH-decreasing agents.

In another preferred embodiment of the method, treatment comprising glucose deprivation or hypoxia or radiation is administered in conjunction with the GSH decreasing agents.

In another preferred embodiment of the method, a cytotoxic agent is administered in conjunction with the GSH-decreasing agent or agents, wherein the cytotoxic agent is preferably selected from the group of cytotoxic agents listed in Table 1.

Another embodiment of the invention is a composition comprising a carrier and a pharmaceutically effective amount of a GSH-decreasing agent. In different embodiments, the GSH-decreasing agent contains or does not contain an aliphatic side chain.

In another embodiment of the invention, the GSH-decreasing agent containing the aliphatic side chain in the composition is a first GSH-decreasing agent and the composition also comprises a second GSH-decreasing agent, and the combined amount of GSH-decreasing agents is a pharmaceutically effective amount, and

preferably the first GSH-decreasing agent harbors an aliphatic side chain and the second GSH-decreasing agent does not harbor an aliphatic side chain; most preferably, the first GSH-decreasing agent harboring the aliphatic side chain is selected from the group consisting of tocopherol quinones, preferably gamma-tocopherol quinone, delta- tocopherol quinone and coenzyme Q (ubiquinone) and the second GSH-decreasing agent which does not harbor an aliphatic side chain is selected from the group consisting of those chemicals which do not harbor a aliphatic side chain which are listed in Table 2 and alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

In another preferred embodiment of the invention, the first and second GSH-decreasing agents in the composition are from the adduct formation class of GSH-decreasing agents, preferably selected from the group consisting of the chemicals listed in Table 2.

Another preferred embodiment of the invention is a composition which comprises a third GSH-decreasing agent, wherein the third GSH-decreasing agent is preferably from the oxidation class of GSH-decreasing agents, most preferably selected from the group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

Another preferred embodiment of the invention is a composition which comprises a fourth GSH-decreasing agent, wherein the third and fourth GSH-decreasing agent are preferably from the oxidation class of GSH decreasing agents, most preferably selected from the group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

A preferred embodiment of the invention is a composition of one or more GSH-decreasing agents wherein at least one of the GSH-decreasing agents is selected from the group consisting of foods, spices and vitamins, preferably selected from the group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone. In other preferred embodiments of the composition, at least two, at least three and at least four of the GSH-decreasing agents are selected from

the group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

A preferred embodiment of the invention is a composition wherein one of the GSH-decreasing agents is buthionine sulfoximine or cycloheximide.

Another preferred embodiment of the invention additionally comprises a cytotoxic agent. Another preferred embodiment of the invention is a drug delivery system comprising at least one composition comprising a carrier and an amount of a GSH-decreasing agent wherein the combined amount of the GSH-decreasing agent is a pharmaceutically effective amount. In different embodiments, the GSH-decreasing agent contains or does not contain an aliphatic side chain.

In another preferred embodiment of the invention the drug delivery system has a first GSH-decreasing agent and also a separate second GSH-decreasing agent, and the combined amount of GSH-decreasing agents is a pharmaceutically effective amount, and preferably the first GSH-decreasing agent harbors an aliphatic side chain and the second GSH-decreasing agent does not harbor an aliphatic side chain, and most preferably the first GSH-decreasing agent is selected from the group consisting of gamma- tocopherol quinone, delta- tocopherol quinone and coenzyme Q (ubiquinone) and the second GSH-decreasing agent is selected from the group consisting of those chemicals which do not harbor a aliphatic side chain which are listed in Table 2 and alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

In another preferred embodiment of the invention, the drug delivery system comprises a separate third GSH-decreasing agent, preferably from the oxidation class of GSH-decreasing agents, most preferably selected from the group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

In another preferred embodiment of the invention, the drug delivery system comprises a separate fourth GSH-decreasing agent, wherein the third and fourth GSH-decreasing agents are preferably from the oxidation class of GSH decreasing agents, most preferably selected from the group consisting of alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

In another preferred embodiment of the invention, at least one of the GSH-decreasing agents in the drug delivery system is selected from the group consisting of foods, spices and vitamins, preferably selected from the group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone. In other preferred embodiments of the composition, at least two, at least three, and at least four of the GSH-decreasing agents are selected from the group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

In another preferred embodiment of the invention, at least one of the GSH-decreasing agents in the drug delivery system is buthionine sulfoximine or cycloheximide.

In another preferred embodiment of the invention the drug delivery system additionally comprises buthionine sulfoximine or cycloheximide.

In another preferred embodiment of the invention the drug delivery system additionally comprises a cytotoxic agent, preferably selected from the group of cytotoxic agents listed in Table 1.

Also contemplated is the use of one or more GSH-decreasing agents in the manufacture of a composition or medicament having anti-tumor activity substantially as described in the specification.

EXAMPLE 4

EXPERIMENTS FOR SUPPORTING THE PRESENT INVENTION

The following experiments are performed using animals such as rats having a tumor in various parts of the body. Control: 20 animals; Test: 20 animals. Evaluate control and test animals until most of the control animals die. Then sacrifice both remaining control and test animals and evaluate tumors. The results show that tumors in test animals either have not grown (cell cycle arrest) or have shrunk (apoptosis), relative to tumors in control animals.

A. Non-metastasized Tumor

Test animals: Introduce 10 or 20 or 50 mg/Kg/day of alpha-lipoic acid and 10 or 20 or 50 mg/Kg/day of curcumin.

B. Metastasized Tumor

Test animals: Add 10 or 20 or 50 mg/Kg/day each of tocopherol, quinone, curcumin and alpha-lipoic acid.

C. Metastasized Tumor

Test animals: Same as (B), plus 10 or 20 or 50 mg/Kg/day of ubiquinone

D. Metastisized Tumors

Test animals: Add 10 or 20 or 50 mg/Kg/day of vitamin K, ubiquinone, curcumin, soy isoflavones, ferenitide

E. Test animals : Add BSO and or cycloheximide (about 1 microM) to A) through (D) where the concentration of the GSH-decreasing agents in the various examples varies from 1-50 mg/Kg/day.

Alternatively, deprive the animals of glucose together with (A) through (D), where the concentration of the GSH-depleting agents in the various examples varies from 1-50 mg/Kg/day.

F. Test animals: Add standard chemotherapeutic agents (in a concentration of 0.1 - 5 µM) such as melphelan to (A) through (D), where the concentration of the GSH-depleting agents in the various examples varies from 1-50 mg/Kg day.

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What is claimed is:

1. A method of treating a tumor in a subject, comprising administering a pharmaceutically effective amount of a GSH-decreasing agent to the subject.

2. The method of claim 1 where said GSH-decreasing agent contains a aliphatic side chain.

3. The method of claim 1 where said GSH-decreasing agent does not contain a aliphatic side chain.

4. The method of claim 2 wherein said GSH-decreasing agent containing said aliphatic side chain is a first GSH-decreasing agent and wherein a second GSH-decreasing agent is administered in conjunction with said first GSH-decreasing agent, and wherein said combined amount of GSH-decreasing agents is a pharmaceutically effective amount.

5. The method of claim 4 wherein said first GSH-decreasing agent harbors a aliphatic side chain and said second GSH-decreasing agent does not harbor a aliphatic side chain.

6. The method of claim 4 wherein said first GSH-decreasing agent harboring said aliphatic side chain is selected from said group consisting of alpha -tocopherol quinone, gamma- tocopherol quinone, delta- tocopherol quinone and coenzyme Q (ubiquinone) and said second GSH-decreasing agent which does not harbor a aliphatic side chain is selected from said group consisting of those chemicals which do not harbor a aliphatic side chain which are listed in Table 2 and alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

7. The method of claim 4 wherein said first and second GSH-decreasing agents are from said adduct formation class of GSH-decreasing agents.

8. The method of claim 7 wherein said first and second GSH-decreasing agents from said adduct formation class of GSH-decreasing agents are selected from the group consisting of said chemicals listed in Table 2 and examples.

9. The method of claim 4 wherein a third GSH-decreasing agent is administered in conjunction with said first and second GSH-decreasing agents.

10. The method of claim 9 wherein said third GSH-decreasing agent is from said oxidation class of GSH-decreasing agents.

11. The method of claim 10 where said third GSH-decreasing agent from said oxidation class of GSH-decreasing agents is selected from said group consisting of alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

12. The method of claim 7 wherein a third GSH-decreasing agent is administered in conjunction with said first and second GSH-decreasing agents.

13. The method of claim 12 wherein said third GSH-decreasing agent is from said oxidation class of GSH-decreasing agents.

14. The method of claim 13 where said third GSH-decreasing agent of said oxidation class of GSH-decreasing agents is selected from said group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

15. The method of claim 9 wherein a fourth GSH-decreasing agent is administered in conjunction with said first, second and third GSH-decreasing agents.

16. The method of claim 15 wherein said third and fourth GSH-decreasing agent are from said oxidation class of GSH decreasing agents.

17. The method of claim 16 wherein said third and fourth GSH-decreasing agent from said oxidation class of GSH decreasing agents are selected from said group consisting of alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine {auto-oxidized}, oxidized low density lipoproteins (LDLs), quinones and duraquinone.

18. The method of claim 12 wherein a fourth GSH-decreasing agent is administered in conjunction with said first second and third GSH-decreasing agents.

19. The method of claim 18 wherein said third and fourth GSH-decreasing agent are from said oxidation class of GSH decreasing agents.

20. The method of claim 19 wherein said third and fourth GSH-decreasing agent from said oxidation class of GSH decreasing agents are selected from said group consisting of alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs, quinones and duraquinone).

21. The method of claim 19 wherein at least one of said GSH-decreasing agents is selected from said group consisting of foods, spices and vitamins.

22. The method of claim 21 wherein said GSH-decreasing agent from said group consisting of foods, spices and vitamins is selected from said group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

23. The method of claim 22 wherein at least two of said GSH-decreasing agents are selected from said group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

24. The method of claim 23 wherein at least three of said GSH-decreasing agents are selected from said group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

25. The method of claim 24 wherein all of said GSH-decreasing agents are selected from said group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

26. The method of claim 4, 9 or 15 wherein one of said GSH-decreasing agents is buthionine sulfoximine or cycloheximide.

27. The method of claim 4 or 9 wherein buthionine sulfoximine or cycloheximide is administered in conjunction with said GSH-decreasing agents.

28. The method of claim 15 wherein buthionine sulfoximine or cycloheximide is administered in conjunction with said GSH-decreasing agents.

29. The method of claim 4 wherein treatment comprising glucose deprivation or hypoxia or radiation is administered in conjunction with said GSH decreasing agents.

30. The method of claim 9 wherein treatment comprising glucose deprivation or hypoxia or radiation is administered in conjunction with said GSH decreasing agents.

31. The method of claim 15 wherein treatment comprising glucose deprivation or hypoxia or radiation is administered in conjunction with said GSH decreasing agents.

32. The method of claim 1 wherein a cytotoxic agent is administered in conjunction with said GSH-decreasing agent.

33. The method of claim 32 wherein said cytotoxic agent is selected from said group of cytotoxic agents listed in Table 1.

34. The method of claim 4 wherein a cytotoxic agent is administered in conjunction with said GSH-decreasing agents.

35. The method of claim 34 wherein said cytotoxic agent is selected from said group of cytotoxic agents listed in Table 1.

36. The method of claim 9 wherein a cytotoxic agent is administered in conjunction with said GSH-decreasing agents.

37. The method of claim 36 wherein said cytotoxic agent is selected from said group of cytotoxic agents listed in Table 1.

38. The method of claim 15 wherein a cytotoxic agent is administered in conjunction with said GSH-decreasing agents.

39. The method of claim 38 wherein said cytotoxic agent is selected from said group of cytotoxic agents listed in Table 1.

40. The method of any of claims 1-39 wherein said pharmaceutically effective amount of said GSH-decreasing agent administered to said subject is in an amount of from about 0.1 to about 50 mg per Kg body weight per day.

41. The method of claim 40 wherein said amount of said GSH-decreasing agent administered is in an amount of from about 10 to about 45 mg per Kg body weight per day.

42. The method of claim 41 wherein said pharmaceutically effective amount of said GSH-decreasing agent administered to said subject is in an amount of from about 20 to about 40 mg per Kg body weight per day.

43. A composition for use in controlling a proliferative behavior of a cell, the composition comprising a carrier and a pharmaceutically effective amount of a GSH-decreasing agent.

44 The composition of claim 43 where said GSH-decreasing agent contains an aliphatic side chain .

45. The composition of claim 44 where said GSH-decreasing agent does not contain an aliphatic side chain.

46. The composition of claim 44 wherein said GSH-decreasing agent containing said aliphatic side chain is a first GSH-decreasing agent and which also comprises a second GSH-decreasing agent, and wherein said combined amount of GSH-decreasing agents is a pharmaceutically effective amount.

47. The composition of claim 46 wherein said first GSH-decreasing agent harbors an aliphatic side chain and said second GSH-decreasing agent does not harbor an aliphatic side chain.

48. The composition of claim 47 wherein said first GSH-decreasing agent harboring said aliphatic side chain is selected from said group consisting of gamma- tocopherol quinone, delta- tocopherol quinone and coenzyme Q (ubiquinone and said second GSH-decreasing agent which does not harbor an aliphatic side chain is selected from said group consisting of those chemicals which do not harbor a aliphatic side chain which are listed in Table 2 and alpha-lipoic acid, hydrogen peroxide , ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

49. The composition of claim 46 wherein said first and second GSH-decreasing agents are from said adduct formation class of GSH-decreasing agents.

50. The composition of claim 49 wherein said first and second GSH-decreasing agents from said adduct formation class of GSH-decreasing agents are selected from said group consisting of said chemicals listed in Table 2.

51. The composition of claim 47 which comprises a third GSH-decreasing agent.

52. The composition of claim 51 wherein said third GSH-decreasing agent is from said oxidation class of GSH-decreasing agents.

53. The composition of claim 52 where said third GSH-decreasing agent of said oxidation class of GSH-decreasing agents is selected from said group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

54 The composition of claim 47 wherein said composition comprises a fourth GSH-decreasing agent.

55. The composition of claim 54 wherein said third and fourth GSH-decreasing agent are from said oxidation class of GSH decreasing agents.

56. The composition of claim 55 wherein said third and fourth GSH-decreasing agent from said oxidation class of GSH decreasing agents are selected from said group consisting of alpha-lipoic acid, hydrogen peroxide, ascorbic acid (auto-oxidized), dopamine (auto-oxidized), oxidized low density lipoproteins (LDLs), quinones and duraquinone.

57. The composition of any of claims 43-56 wherein at least one of said GSH-decreasing agents is selected from said group consisting of foods, spices and vitamins.

58. The composition of claim 57 wherein at least one of said GSH-decreasing agents is selected from said group consisting of iso-flavones, green tea phenols, curcumin, alpha-lipoic acid and ubiquinone.

59. The composition of any of claims 43-58 wherein at least one GSH-decreasing agent is buthionine sulfoximine or cycloheximide.

60. The composition of any of claims 43-59, additionally comprising a cytotoxic agent

61. A drug delivery system comprising at least one composition comprising a carrier and an amount of a GSH-decreasing agent according to any of claims 1-60 wherein said combined amount of said GSH-decreasing agent is a pharmaceutically effective amount.

62. A composition for controlling a proliferative behavior of a cell in a subject, comprising an agent for increasing a redox potential (E) of the cell, the agent being administratable to the subject.

63. The composition of claim 62, wherein said agent is a GSH-decreasing agent.

64. The composition of claim 63, wherein said GSH-decreasing agent is said agent of any of claims 43-59.

65. The composition of any of claims 63-64, wherein a solubility of said GSH-decreasing agent is adjusted according to a location of the proliferative behavior of the cell in the subject.

66. The composition of any of claims 63-65, further comprising at least a second GSH-decreasing agent, wherein at least one GSH-decreasing agent forms an adduct with GSH and at least a second GSH-decreasing agent oxidizes GSH.

67. A method for controlling a proliferative behavior of a cell in a subject, comprising administering an agent for increasing a redox potential (E) of the cell to the subject.

68. The method of claim 67, wherein said agent is a GSH-decreasing agent.

69. The method of claim 68, wherein said GSH-decreasing agent is said agent of any of claims 43-59.

70. A composition for treating a malignancy in a subject, comprising an agent for increasing a redox potential (E) of the cell, the agent being administratable to the subject.

71. The composition of claim 70, wherein said agent is a GSH-decreasing agent.

72. The composition of claim 71, wherein said GSH-decreasing agent is said agent of any of claims 43-59.

73. The composition of any of claims 70-72, wherein the malignancy is characterized by slow proliferation of cells of the malignancy.

74. A method for treating a malignancy in a subject, comprising administering an agent for increasing a redox potential (E) of the cell to the subject.

75. The method of claim 74, wherein said agent is a GSH-decreasing agent.

76. The method of claim 75, wherein said GSH-decreasing agent is said agent of any of claims 43-59.

77. The method of any of claims 74-76, wherein the malignancy is characterized by slow proliferation of cells of the malignancy.

78. A composition for treating a disease in a subject, the disease being characterized by apoptosis, the composition comprising an agent for decreasing a redox potential (E) of the cell, the agent being administratable to the subject.

79. A method for treating a disease in a subject, the disease being characterized by apoptosis, the method comprising administering an agent for decreasing a redox potential (E) of the cell to the subject.

80. The method of claim 79, wherein said agent comprises a GSH-increasing agent, wherein said GSH-increasing agent includes N-acetylcysteine (NAC).

81. A method for treating a disease in a subject, the disease being characterized by apoptosis, the method comprising injecting an agent for decreasing a redox potential (E) of cells of the diseased tissue directly into the tissue.

82. A method for increasing GSH in a subject, comprising administering an anti-oxidant to the subject.

Table 1a Anti-Cancer Agents

Group	Examples	Mechanism
Plant Alkaloids		
Vinca	Vincristine Vinblastine	Inhibition of microtubular assembly
Mandrake	Podophyllotoxin	
	VP-16 (Etoposide) VM-26	DNA Breakage
Quinoline alkaloids	Topetecan Camptosar (Irinotecan)	Anti DNA topoisomerase I (S phase)
Antibiotics		
Anthracyclins	Doxorubicin Daunorubicin	Intercalation of DNA Free radical formation
Others	Bleomycin	DNA breakage
	Mitomycin C	DNA alkylation & cross-linking
	Actinomycin D	DNA binding by intercalation
Antimetabolites		Inhibition of DNA synthesis
Antifolates	Methotrexate (MTX)	Dihydrofolate Reductase inhibition
Fluoropyrimidines	5-Fluorouracil (5FU)	
Arabinose nucleosides	Cytarabine (Ara C) Ara A	DNA polymerase inhibition
Purine analogues	6-Mercaptopurin	Incorporation into DNA
Hydoxyurea		
Alkylating agents		Alkylation = formation of covalent bond with DNA
Conventional	Nitrogen mustard (Mechlorethamine) Cyclophosphamide Ifosfamide Melphelan Chlorambucil Busulphan	
Nitrosureas	Bis-chloroethylnitrosurea (BCNU) 1-(2chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) 1-(2-chloroethyl)-3-(4-trans-methylcyclohexyl)-1-nitrosourea (MeCCNU)	In addition to alkylation, they form isocyanates, which deplete GSH, inhibit DNA repair and alter editing of DNA
Heavy metal compounds	Cisplatin	Change in DNA conformation Inhibtion of DNA synthesis
	Trisenox (arsenic trioxide)	Induces caspase proenzyme expressions & activates caspases. Induces apoptosis and differentiation of APL cells
Methylmelamines	Hexamethylmelamine Pentamethylmelamine	Uncertain
Carbazines	Procarbazine Dacarbazine	Uncertain, possibly alkylation
Enzyme	Asparaginase	Depletion of asparagine in tumor cells

Table 1b New Anti-Cancer Agents

Group	Examples	Mechanism
Hormone antagonists		
Anti-Estrogen	Tamoxifen Arimidex (Anastrozole) Aromasin (Exemestane)	Prevent binding of estrogen to receptors
	Formestane Letrozole	Inhibit aromatase, leading to reducing in estrogen
	Medroxyprogesterone	
Anti-Androgen	[Orchiectomy]	
Estrogens	Diethylstilbestrol	
LHRH agonists	Leuprorelin Goserelin Buserelin	Inhibit testosterone synthesis
Pure antiandrogens	Cyproterone Flutamide Bicalutamide Magestrol acetate Ketoconazole Aminoglutethimide	Prevent binding of testosterone to receptors
Quinoline alkaloids	Topetecan Camptosar (Irinotecan)	Anti DNA topoisomerase I (S phase)
Taxoids	Taxotere (Docetaxel) Taxol (Paclitaxel)	Block disassembly of micro-tubules, preventing cancer cells from dividing
Cyclin-dependent kinase inhibitors	Flavopiridol	Causes cell cycle arrest at G_1 or G_2
		Blocks ATP binding site
		Induces apoptosis
Matrix metalloproteinase inhibitors	COL-3 (6-deoxy-6-demethyl-4-dedimeth ylamino-tetracycline)	Interfere with several aspects of MMP expression and activation and inhibit tumor growth and metastases
Angiogenesis inhibitors	Angiostatin Endostatin Semoxind	Inhibit angiogenesis
Retinoids	All-trans retinoic acid 9-Cis retinoic acid	Induce differentiation and growth arrest of neuroblastoma
Heavy metal compounds	Trisenox (arsenic trioxide)	Induces caspase proenzyme expressions & activates caspases. Induces apoptosis and differenti-ation of APL cells
Biological Response Modifiers		
Monoclonal antibodies	Rituxan (Rituximab)	Attaches to CD20 on surface of lymphoma cells; triggers apoptosis
Cytokines	Interferon-alpha	? direct attack or via immune regulation

Table 2. Possible Aliphatic and non-Aliphatic GSH-depleting Agents

Anthraquinone
Butyric acid (Butanoic acid) Phenylbutyrate
Camptothecin
Capsaicin
Catecholestrogens
Catechin Epicatechin
Cinnamaldehyde
Cinnamic acid
Curcumin
Daidzen
Dauorubicin (Daunomycin)
4'-Demethyl epi- podophyllotoxin
Dexamethasone
Diarylheptanoids Yakuchinone A Yakuchinone B
Diethyl maleate (DEM)
Dicoumarol
Doxorubicin (Adriamycin)
Epigallocatechin gallate
Epothilones A, B
Erbstatin
Estradiol
Flavopiridol
Gemcitabine
Geinstein
ß-Lapachone
Mifepristone
Phenols
Phytol
Quinones Naphthoquinones Dunninione
Retinamide
Retinoic acid
Rotenone
Resveratrol
Staurosporine